

AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions, and listings, of claims in the application:

Listing of the claims:

1. (Currently Amended) A process for decreasing a level of aggregate of pegylated protein isoforms, wherein the pegylated protein is a growth hormone antagonist, said process comprising the steps of:
 - (a) providing said pegylated protein isoforms; and
 - (b) separating said pegylated protein isoforms by anion exchange chromatography using an anion exchange resin under sufficient conditions to decrease said level of said aggregate, and wherein said level of said aggregate is less than 6% by weight based on the total weight of said isoforms and said aggregate, and wherein said pegylated protein isoforms comprises one or more of the isoforms PEG-1, PEG-2, PEG-3, PEG-4, PEG-5, PEG-6, PEG-7, PEG-8, and PEG-9 and any aggregate, trisulfide impurity and des-phe impurity thereof.
2. (Previously Amended) The process of claim 1 further comprising the step (a1) of pegylating unpegylated or a partially pegylated form of the protein, or pegylating both to provide said pegylated protein isoforms.
3. (Original) The process of claim 2 wherein said pegylating step (a1) comprises pegylating with free PEG selected from the group consisting of PEG-N-hydroxysuccinimide-5K, PEG-succinimidyl carbonate-5K, PEG-succinimidyl propionate-5K, PEG2-maleimide-40K (2 x 20K), PEG2-N-hydroxysuccinimide-40K (2 x 20K), PEG2-aldehyde-40K (2 x 20K).
4. (Original) The process of claim 3 wherein a stoichiometric weight ratio of said free PEG to said unpegylated protein is from about 0.5 to about 100.

5. (Original) The process of claim 4 wherein said stoichiometric weight ratio is from 1.5 to about 2.5.

Claims 6-7 (Canceled)

8. (Original) The process of claim 2 wherein said pegylating step (a1) is conducted at pegylating pH from about 3 to about 10.

Claims 9-11 (Canceled)

12. (Previously Presented) The process of claim 8 wherein said pegylated pH is from about 7.40 to about 7.80.

Claims 13-14 (Canceled)

15. (Previously Presented) the process of claim 2 wherein said pegylation step (a1) is conducted at a pegylating temperature from about 18 to about 25° C.

16. (Currently Amended) The process of claim 1 further comprising an optional hydrophobic interaction chromatography (HIC) step of selecting said pegylated protein by (HIC) using an HIC resin.

17. (Currently Amended) the process of claim 2 further comprising an optional hydrophobic interaction chromatography (HIC) step (a2) selecting said pegylated protein by (HIC) using an HIC resin.

Claims 18-19 (Canceled)

20. (Previously Presented) The process of claim 17 wherein said HIC load is less than or equal to about 4.1 g protein/L of packed bed-volume of HIC resin.

Claims 21-22 (Canceled)

23. (Original) The process of claim 17 wherein said HIC step (a2) is conducted at HIC temperature from about 10 to about 40 °C.

Claims 24-25 (Canceled)

26. (Previously Presented) The process of claim 17 further comprising a ultrafiltering/diafiltering (UF/DF#3) step (a3) ultrafiltering/diafiltering (UF/DF#3) of an eluent from said HIC step (a2).

Claims 27-30 (Canceled)

31. (Original) The process of claim 1 wherein said step (b) further comprises a step (b1) of loading said pegylated protein including any impurity and any aggregate thereof on said anion exchange (AEX) resin to provide loaded pegylated protein.

Claims 32-33 (Canceled)

34. (Original) The process of claim 31 wherein said step (b1) is conducted at an AEX loading conductivity of less than or equal to about 10 mS/cm.

Claims 35-36 (Canceled)

37. (Original). The process of claim 31 wherein said step (b1) is conducted at a loading pH from about 5 to about 10.

Claims 38-39 (Canceled)

40. (Original) The process of claim 31 wherein said step (b1) is conducted at an AEX load of pegylated protein including any impurity or said aggregate thereof of less than or equal to about 10 g protein/L of packed bed-volume of AEX resin.

Claims 41-42 (Canceled)

43. (Currently Amended) A process for decreasing a level of aggregate of pegylated protein isoforms, wherein the pegylated protein is a growth hormone antagonist, said process comprising the steps of:

(a) providing said pegylated protein isoforms; and

(b) separating said pegylated protein isoforms by anion exchange chromatography using an anion exchange resin under sufficient conditions to decrease said level of said aggregate, and wherein said level of said aggregate is less than 6% by weight based on the total weight of said isoforms and said aggregate, The process of claim 1 and wherein said pegylated protein comprises one or more of said pegylated protein isoforms PEG-1, PEG-2, PEG-3, PEG-4, PEG-5, PEG-6, PEG-7, PEG-8, and PEG-9 and any aggregate, trisulfide impurity and des-phe impurity thereof and any unpegylated impurity of said protein and any free PEG molecules.

Claims 44-53 (Canceled)

54. (Previously Presented) The process of claim 1 further comprising a pooling step (c) of pooling discrete amounts of said pegylated protein isoforms to yield a pooled pegylated protein by a technique selected from the group consisting of capillary electrophoresis (CE), sodium dodeCyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), hydrophobic interaction chromatography (HIC), anion exchange (AEX) chromatography, cation exchange (CEX) chromatography, reverse-phase high pressure liquid chromatography (RPHPLC), size exclusion high pressure liquid chromatography (SEHPLC), affinity chromatography (AC) and combinations thereof.

Claims 55-204 (Canceled)

205. (Previously Presented) The process of claim 1 wherein said growth hormone antagonist is a human growth hormone antagonist.

206. (Currently Amended) A process for decreasing a level of aggregate of pegylated protein isoforms, wherein the pegylated protein is a growth hormone antagonist, said process comprising the steps of:

(a) providing said pegylated protein isoforms; and
(b) separating said pegylated protein isoforms by anion exchange chromatography using an anion exchange resin under sufficient conditions to decrease said level of said aggregate, and wherein said level of said aggregate is less than 6% by weight based on the total weight of said isoforms and said aggregate, and ~~The process of claim 1~~ wherein said human growth hormone antagonist is B-2036.

207. (Previously Presented) The process of claim 206 wherein said human growth hormone antagonist comprises an amino acid sequence of SEQ ID NO:1.

208. (Currently Amended) The process of claim 1 54 wherein said growth hormone antagonist is a human growth hormone antagonist.

209. (Previously Presented) The process of claim 208 wherein said human growth hormone antagonist is B-2036.

210. (Previously Presented) The process of claim 209 wherein said human growth hormone antagonist comprises an amino acid sequence of SEQ ID NO:1.

211. (Previously Presented) The process of claim 54 wherein said pooled pegylated protein comprises isoforms PEG-4, PEG-5, and PEG-6.

212. (Previously Presented) The process of claim 54 wherein said pooled pegylated protein comprises at least 90% by weight based on a total weight of PEG-1, PEG-2, PEG-3, PEG-4, PEG-5, PEG-6, PEG-7, PEG-8 and PEG-9 pegylated protein isoforms and any aggregate thereof.

213. (Previously Presented) The process of claim 54 wherein said pooled pegylated protein comprises at least 94% by weight based on a total weight of PEG-1, PEG-2,

PEG-3, PEG-4, PEG-5, PEG-6, PEG-7, PEG-8 and PEG-9 pegylated protein isoforms and any aggregate thereof.

214. (Previously Presented) The process of claim 54 wherein said pooled pegylated protein consists essentially of isoforms PEG-3, PEG-4, PEG-5, PEG-6, and PEG-7.

215. (Previously Presented) The process of claim 54 wherein said pooled pegylated protein consists essentially of isoforms PEG-4, PEG-5, and PEG-6.

216. (Previously Presented) The process of claim 1 wherein said level of aggregate is less than or equal to about 5% by weight of the pegylated protein.

217. (Previously Presented) The process of claim 1 wherein said level of aggregate is less than or equal to about 2% by weight of the pegylated protein.

218. (New) The process of claim 205 wherein said human growth hormone antagonist is B-2036.